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WORKING MATERIAL

CONSULTANTS GROUP MEETING ON

GENETIC SEXING AND POPULATION GENETICS OF SCREWWORMS

VIENNA, AUSTRIA

7 - 11 AUGUST 2000

CONTENTS

1. EXECUTIVE SUMMARY

A Thematic Plan on SIT for Screwworms developed in 1999 by IPC and TC identified certain R and D bottlenecks to the expansion of this technology into new agricultural areas. This consultant's meeting was held to review these conclusions and to advise the Agency on the need, or otherwise, of initiating a CRP to address the bottlenecks identified in the Thematic Plan. None of the consultants (Annex 1) participated in the preparation of the Thematic Plan.

In 2001 it is expected that the New World Screwworm, *Cochliomyia hominivorax*, will have been eradicated from all of Central America, including Panama where a sterile release barrier will be established to prevent re-invasion from South America. This barrier will need to be maintained indefinitely with its associated costs. The use of an all-male strain in the production facility would have very positive impact on the cost/benefit analysis of the programme. The Director of the Screwworm Programme in Central America made this point very strongly during the Thematic Plan discussions and at a subsequent technical meeting in Tuxtla Gutierrez (see Annexes 2 and 3). Interest to expand the programme into South America is now being shown by certain countries in the region where the economic feasibility of implementing an SIT programme might depend on producing sterile flies more economically and here again the use of a genetic sexing strain could play an important role. For the Old World Screwworm, *Chrysomya bezziana* the Australian authorities have just completed a successful small field trial of the SIT in Malaysia and it is proposed that more extensive field tests be carried out in the region.

For both the New World Screwworm in South America and the Old World Screwworm, in Asia there is virtually no information regarding the population structure in relation to the implementation of an SIT programme. Is the Old World Screwworm a single species over its very wide distribution and are the populations of New World Screwworm in South America the same as in Central America and related to each other? Are the populations isolated? These questions can to a large extent be answered using modern molecular techniques of DNA analysis and answers to these questions would provide support for decision makers in the planning and implementation of new SIT programmes.

The consultant's reviewed work on both of these subjects during their presentations (Annexes 4 and 5) and discussed if a CRP was the appropriate vehicle to address the problems. Any progress in these areas will be dependent on the creation of a network of scientists and a CRP would be an ideal mechanism for this. The consultants also identified other partners who could interact in a positive way with any new CRP. The number of scientists currently active in the identified areas is not large and it will be important to recruit new people to the field. USDA has already made efforts in this direction both in ARS and APHIS. The consultants concluded that a viable and competent group of scientists could be identified to form the nucleus of a new CRP. A prioritized list of research activities for the CRP was formulated together with relevant institutions and partners.

Having decided that a CRP would be an appropriate mechanism to address the R and D problem areas, the consultants developed a draft logical framework and this will be submitted through the appropriate channels to the Research Contracts sub-committee.

2. BACKGROUND

The New World Screwworm (NWS), *Cochliomyia hominivorax*, and the Old World Screwworm (OWS), *Chrysomya bezziana*, are major parasitic insect pests that profoundly affect livestock, and therefore, the economic development of the agricultural sector in major parts of the world (FAO 1992, Spradbery 1993). The disease caused by the infestation of living vertebrate tissue by the larvae of screwworm flies is called myiasis. The magnitude of the cutaneous myiasis problem dictates that its control be a prerequisite to the maintenance of a viable livestock industry and the increasing need for agricultural production. The disease can also profoundly affect humans, pets, and wildlife.

The current distribution of NWS includes part of the Caribbean (Cuba, Dominican Republic, Haiti, Jamaica, Trinidad and Tobago) and all of South America (with the exception of Chile) (Wyss & Galvin 1996). The current distribution of OWS extends in an arc from South Africa through the Middle East, then through Southern China, the Philippines, Indonesia and East as far as Papua New Guinea (Brown *et al*. 1998).

The impact of screwworm flies on the livestock sector is influenced by husbandry practices. Economic losses are important where extensive farming systems are practiced and animals are not closely supervised to identify the need for early treatment. In smallholder farming systems, control procedures such as intensive animal inspection and treatment of wounds with insecticides are applicable but involve significant recurrent expenditures. Both systems, therefore, warrant labor-intensive interventions that carry high financial and productivity costs.

The aims of the veterinary services of the affected countries are focused on the development of sustainable animal agriculture and food security. The incidence and severity of the disease are modulated by existing local conditions such as:

- Livestock population, distribution, density and husbandry procedures.
- Commercial movement of animals.
- Wildlife population and their migratory habits.
- Human population density, socio-economic conditions and the public health service.
- Climate and geography.

In addition to direct losses and the financial cost of control, cutaneous myiasis indirectly affects:

- Human health, through protein deficiencies caused by shortage of meat and milk.
- Livestock production, since it causes morbidity and mortality.
- Agricultural production, through the lack of draught animals and manure.
- Rural economy, by preventing integrated agriculture and livestock production.
- National economy, through essential import of living animals and their products.
- Environment, though the use of insecticides.

3. SCREWWORM SIT

The United States Department of Agriculture, in collaboration with Mexico and the other host countries throughout Central America, has successfully eradicated NWS from North and Central America, with the exception of Panama where a programme is currently being completed. A programme is also being implemented in Jamaica. Annual animal producer benefits have been estimated at $853 million for the United States, $314 million for Mexico and $87.8 million for Central America (Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama) (personal communication, J. H. Wyss).

Unfortunately, no economic data are available for OWS in endemic countries. Studies in Australia have estimated producer losses of AUD $281 million per year if OWS became established in that country (Anaman *et al*. 1993). Also, ongoing studies sponsored by IAEA are in place to estimate losses in Malaysia and islands of the Philippines.

In the case of NWS, the substantial economic costs involved clearly justify the eradication of the disease from the endemic areas and the prevention and rapid response to invasions into screwworm-free areas. The development of the Sterile Insect Technique (SIT) in the 1950's created a revolution in the methods available to control major insect vectors of animal diseases and now forms the basis for NWS national and regional eradication programs in the Americas. The SIT has proven to be a promising technology for the control and eradication of OWS but requires development of more competitive strains.

The SIT is often the only tool available to achieve the eradication of major insect pest populations in an environmentally friendly way. It consists of the systematic release, on an area wide basis, of sterile insects as the final component of a technological package involving surveillance, suppression and regulatory measures.

The SIT component involves the mass production of the target insect pest (NWS or OWS) followed by sterilization and release. When sterile males mate with fertile wild females, no progeny are produced. Providing that the ratio of sterile to fertile insects is maintained at a high level within the target area, reproduction in the pest population can be reduced significantly from generation to generation, eventually leading to eradication.

Sterilization is accomplished by exposing insects to a specific dose of radiation. No other methods are available or appropriate to achieving insect sterilization. Nuclear technology has not only a clear comparative advantage in sterilizing mass reared insects, but is, at present, the only technology available for this purpose. Also, nuclear technology is essential to developing mutant markers, chromosome rearrangements, all-male strains and molecular techniques.

Although SIT is a form of genetic control, there have been only a few studies on the genetics of screwworms (for example LaChance & Whitten 1986, Azeredo-Espin 1993, Strong & Mahon 1996, Taylor *et al*. 1996, Lessinger *et al*. 2000). In the case of NWS, eradication programs have successfully eliminated the pest population from the mainland areas north of Panama. The maintenance of a barrier to guard against introduction of NWS to screwworm free areas would benefit from the development of genetic sexing strains. Also, future eradication or control efforts involving SIT would require information on genetic variability of the pest populations. For OWS, more detailed genetic information is

necessary before informed decisions can be made on initiating SIT programs (IAEA, 1999).

The cost/benefit ratio of the SIT program would be considerably enhanced by the utilization of an all-male strain used in the mass-production phase of the program. All male strains have been successfully developed in other important pests species and they are used throughout the world in Medfly SIT (Franz 1996; Fisher 2000).

4. PARTNERSHIPS IN SCREWWORM SIT AND A NEW CRP

The IAEA is the leading technical organization supporting the development and application of SIT. It seeks to collaborate with other partners on co-ordinated efforts aimed at eradicating and controlling screwworm in Member States.

Lead agencies for overall NWS program activities are the US Department of Agriculture (USDA) and the Food and Agriculture Organization of the United Nations (FAO), and the International Atomic Energy Agency (IAEA) for SIT. To assist these activities, two Support Centers were identified. These are: the Mexico–United States Screwworm Commission (COM), in Tuxtla Gutierrez, Chiapas, Mexico, for sterile fly production activities**;** the Panama–United States Commission for the Eradication and Prevention of Screwworms (COPEG), in Panama City, Panama, for other aspects of eradication programs and production of sterile flies in the future.

Lead agencies for overall OWS program activities are FAO, the IAEA for SIT and the Arab Organization for Agricultural Development (AOAD) for program development in Arabic countries. To assist in these activities, two Support Centers were identified. They are: the Institut Haiwan, Kluang-Johor, Malaysia**;** the CSIRO Australia, for sterile fly production**,** biological studies, including field ecology, lures and attractants, and other aspects of eradication programs.

In the Thematic Plan for IAEA Technical Cooperation on Sterile Insect Technique for Old and New World Screwworm (1998), several areas of needed research were identified.

> *We are of the opinion that the best approach to address this applied research in support of future projects is the establishment of a* ***Coordinated Research Project*** *to: 1) develop a genetic sexing strains in New World Screwworm for use in SIT and, 2) use DNA fingerprinting to elucidate the population genetics of the two species of screwworm*

4.1 Overall Objective of the CRP

> **TO ENHANCE THE EFFICIENCY OF THE IMPLEMENTATION OF SIT FOR SCREWWORMS AND TO REDUCE RISK ASSOCIATED WITH THE INTRODUCTION OF SCREWWORM INTO NEW AREAS**

Specific Objectives of the CRP

- ***To establish genetic relationships between populations of Old and New World Screwworms.***
- ***To identify the origins of new outbreaks in order to improve quarantine regulations.***
- ***To develop a genetic sexing strain for New World Screwworm.***

Achieving these objectives would be of direct benefit to the expansion of SIT for screwworm through determining the population structure of these pests (are there mating barriers amongst divergent populations?) and in improving the cost/benefit ratio of mass producing sterile males. The beneficiaries would be: 1) livestock, pets, and people in the

endemic areas through reduced trauma from myiasis; 2) livestock producers through increased profitability; 3) the environment through reduced insecticide use; 4) countries free of screwworm by greatly reducing, or possibly eliminating, the risk of introduction of these devastating pests.

We should acknowledge that the two species of screwworm, while presently located separately in the Western and Eastern Hemispheres, might not necessarily stay separated. Movement of people and animals can inadvertently spread both species. Wilful dispersal of these pests is a global concern. Thus, we see an ongoing need to further develop and improve SIT for both species as this technique offers the only known means to eradicate populations of these pests.

4.2 Research Areas

4.2.1. Develop all-male strains for New World Screwworm.

Personnel at the USDA-ARS Midwest Livestock Insects Research Unit (MLIRU) in Lincoln, NE will screen existing colonies for new mutants as well as develop appropriate technology of translocations and cytogenetics for use in developing the genetic sexing strains. Personnel at the USDA-APHIS - Mexico Joint Commission's mass rearing facility in Tuxtla Gutierrez, Mexico will screen for mutants in their existing colonies to be used in the genetic sexing system. Contact will be initiated toward developing collaboration with USDA-ARS in Gainesville, FL to investigate/develop the applicability of transgenic techniques to developing genetic sexing strains of NWS.

4.2.2. Determine the genetic variability of the NWS across its current geographic distribution.

Molecular markers, developed in Brazil and MLIRU, would be used to elucidate the genetic variability in screwworm from different geographic origins. Protocols for sampling, storing samples, DNA extraction and molecular genetic techniques would be distributed (as developed by Brazil and MLIRU) through IAEA channels to collaborators in screwworm endemic areas. An initial workshop would be organized, through IAEA, to establish that molecular genetic techniques are uniformly applied in collaborating countries and that representative samples are maintained for and/or transferred to a central laboratory for molecular genetic studies. DNA sequence analysis will be done to enhance the existing work at Brazil and to deliver additional markers for use in population genetic studies as well as the development of genetic sexing systems.

4.2.3. Determine the genetic variability of the Old World Screwworm across its current geographical distribution.

Evaluate (confirm or otherwise) an East-West divergence of OWS populations indicated by molecular studies (Martin Hall and collaborators). These findings are inconsistent with previous studies and cast doubt on the universality of OWS strains for SIT. Samples collected for studies performed by CSIRO in the early 1990's are still available and the samples should be suitable for molecular analysis. Additional collections would assist in defining the divergence, and in this area we see an opportunity to utilise whatever expertise is available in a range of countries where OWS is a pest. A "central" laboratory should be established to collect, store and analyse material provided by contributors. However, where the expertise and facilities of participants is suitable, local analysis should be encouraged and supported. A workshop would be essential to ensure consistency in

techniques to enable the direct incorporation of intra-country studies into a larger global analysis. Assistance from IAEA is needed to identify potential collaborators interested in participating in an OWS program. Presently, people actively working on OWS, or at least interested in the problem, are known from Malaysia, Iraq, Iran and Australia. Contacts in the Philippines, Indonesia, Thailand, India, and countries in the horn of Africa should be sought in order to establish a network of participants. It would be highly beneficial to this program to interact with the NWS molecular studies program proposed elsewhere in this document. Benefits would accrue from utilising the various probes assembled for use in NWS.

4.2.4. Cryopreservation

We see the development of means to cryopreserve NWS as an important development that will facilitate improvements to SIT in both species. In OWS, the immediate concern is to preserve the existing laboratory adapted strains in Malaysia. Therefore it would be possible to sequentially revive the strains, as well as newly developed strains, for use in competitiveness tests. It is hoped that the cryopreservation techniques developed for NWS are directly applicable to OWS. If this is not the case, further development work will be required.

4.2.5. Development of more competitive strains for Old World Screwworm SIT.

While the efficacy of SIT has been demonstrated in a trial in Malaysia, the sterile males used were poorly competitive. Consequently, large numbers of sterile males were required to achieve significant levels of sterility in the experiment. Using such a strain in an eradication program would prove prohibitively expensive. There are presently two Malaysian colonies that are suitable for mass rearing. A laboratory strain exists in Iraq and we understand that there are plans to establish a new laboratory strain in Iran. Another strain suitable for mass rearing (perhaps from the Philippines) should be established. Strain evaluation could take the form of short field release programs to evaluate competitiveness of sterile flies. In addition, the usefulness of large field cages to compare competitiveness of mass reared flies should be evaluated as a possible means to simplify the development of competitive strains. The Philippines is viewed as a particularly important site for field-testing of new strains, and an IAEA funded cost benefit study is presently underway in that country. It is anticipated that this study will identify that certain islands in the Philippines offer excellent targets for a large trial/eradication demonstration in much the same way as Curacao was used in the early days of the NWS program.

THE ABOVE ITEMS ARE THE MAJOR PRIORITIES REGARDING THE PROPOSED CRP. THE FOLLOWING TWO ITEMS ARE IMPORTANT ISSUES FOR SIT THAT COULD EVOLVE FROM THE NETWORK THAT WILL BE DEVELOPED.

4.2.6. Determine the geographic distribution and population density of NWS in endemic areas in the Caribbean and South America.

Geographic information systems (GIS) and satellite imagery (SI) will be used to predict optimum sampling sites (for example, in Cuba and/or Venezuela). USDA will organize workshops (with assistance from IAEA) so that officials in each country are using standardized protocols for the GIS and SI technology. Existing protocols for sampling screwworms will be used by participants in the Caribbean and South America.

4.2.7. Determine the cost-benefit of conducting SIT in each endemic country.

The basis for any proposed SIT intervention must be a favourable economic analysis of cost/benefits. USDA-APHIS-IS and IAEA could co-ordinate these efforts in countries that express an interest in using this technology.

5. RECOMMENDATIONS

-that the Agency establish a CRP on population genetics and genetic sexing in screwworms, addressing the issues raised above.

-that the Agency, together with USDA-APHIS-IS, conduct a workshop on the standardisation of population sampling of New World Screwworm in South America.

-that efforts be made to fully utilize the Old World screwworm rearing facility in Klung for workshops, training etc

-that suitable locations be identified in S.E. Asia where extensive field trials for Old World Screwworm SIT can be carried out.

-that the Agency initiate economic feasibility studies for New World screwworm SIT in selected countries of South America

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ANNEX 1

Consultants

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ANNEX 2

United States Department of Agriculture	Animal and Plant Health Inspection Service	International Services	Screwworm Prog. Central America Region

April 18, 2000

Dr. Floyd Horn
Administrator
Agriculture Research Service
Jamie L. Whitten Building, 302-A
14 h & Independence Ave., SW
Washington, D.C. 20250

Dear Dr. Horn

A workshop was held on March 29, 2000, in Tuxtla Gutierrez, Mexico, to outline the potential and requirements for developing a genetic sexing, or male-only, strain of the screwworm. The workshop was held in conjunction with the Annual Technical Meeting of the Screwworm Eradication Program and was requested by the USDA, Animal and Plant Health Inspection Service, Screwworm Eradication Program.

We will soon achieve our goal of eradicating screwworm from mainland North America and will maintain a barrier zone in Panama to protect the eradicated areas from reintroduction of this devastating pest. The success of this program has been of extreme economic benefit to the United States, Mexico, and Central America. The planned implementation of a permanent sterile fly barrier in Panama brings with it a long-term funding commitment. The use of a genetic sexing strain would provide tangible benefits through significant reductions in overall cost of the program (reduced costs of materials for fly rearing, cost reductions in fly distribution, and a reduced infrastructure) and potentially a more effective eradication program (improved field performance of sterile male flies). Also, the potential expansion of the program to the Caribbean and South America through the sale of sterile flies will be greatly benefited by more attractive economic projections made possible by a genetic sexing strain.

The example of the success using genetic sexing strains in operational Mediterranean Fruit Fly (Medfly) SIT programs gives us confidence that this can be achieved with screwworm. The initial technical problems associated with the use of these strains have been solved and now almost all Medfly mass rearing facilities use these strains. Key factors leading to the success of the program were the partnerships that evolved during its implementation. Similar partnerships will be required for success in screwworm. To develop an operational genetic sexing strain for the screwworm, a strengthened core research group of geneticists is needed in Lincoln, NE. This would require one full-time classical geneticist to follow the successful model strategy used for the Medfly. A complimentary approach using molecular genetics is also warranted, because of the rapid advances being made in this field. Therefore, the recruitment of a molecular biologist in the program is needed. Without this strengthening of the core group and the commitment to a long-term objective to establish a genetic sexing strain, we see little chance for success. With this core group addressing these needs, we see the potential for research growth by networking with new partners to accelerate the efforts and improved economic potential for the sale of sterile flies.

Respectfully,

John Wyss
Regional Director
USDA-APHIS-IS

Alan Robinson
Workshop Moderator
IAEA/FAO

United States Department of Agriculture | **Agricultural Research Service** | **Office of the Administrator** | **Washington, DC 20250**

JUN 23 2000

SUBJECT: Screwworm Eradication Program

TO: John H. Wyss Regional Director
International Services
Animal and Plant Health Inspection Service

FROM: Floyd P. Horn
Administrator

I want to acknowledge and thank you and Dr. Alan Robinson, Food and Agricultural Organization/International Atomic Energy Agency, for your letter of April 18, 2000, concerning the Animal and Plant Health Inspection Service Screwworm Eradication Program.

In your letter you outlined the need for a strengthened core research group of geneticists in Lincoln, Nebraska, to develop an operational genetic sexing strain for the screwworm. You stated the core group would require one full-time classical geneticist to follow the successful model strategy used for the Medfly and a molecular biologist to address the rapid advances being made in that field.

The Agricultural Research Service (ARS) concurs with your assessment. Dr. Karl Narang, ARS National Program Leader, Animal Pests and Parasites, has already begun discussions with ARS scientists at the ARS Midwest Livestock Insects Research Unit (MLIRU), Lincoln, Nebraska, and the ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida, on the best strategies for accomplishing the male-only strain. The plan will be finalized and implemented as soon as Dr. Phil Scholl, the new Research Leader is on board at MLIRU. ARS is also looking into the possibilities of redeploying its resources at Lincoln, Nebraska, to speed up this effort on the male-only strain.

Should you need additional information, please feel free to contact Dr. Narang at telephone: (301) 504-5771, or E-mail: ksn@ars.usda.gov

cc
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ANNEX 4

Summary of Presentations

1. Molecular Markers to Characterize Genetic Variability in Brazilian Cochliomyia hominivorax.

Dr. Ana Maria L. de Azeredo-Espin, Dept. de Genética e Centro de Biologia Molecular e Eng. Genética, UNICAMP, Campinas -SP, Brasil.

The screwworm fly *Cochliomyia hominivorax* is one of the most important agents of traumatic myiasis throughout neotropical regions. In Brazil this pest is devastating, causing great profit losses for cattle breeders (around U$ 180 million annually). In South America there are no preventive methods to control natural populations of screwworm fly.

The basic knowledge of the genetic variability and evolution within a species is necessary information to understand the structure and evolution of populations. In the case of screwworm populations we are, in our laboratory, conducting analyses with different types of molecular markers in the mitochondrial and nuclear genomes using RFLP, PCR and sequencing procedures and protein electrophoresis to characterize the genetic polymorphism and population structure of screwworms in Brazil. Based on the fragment patterns for the five marker enzymes, 15 mtDNA composite haplotypes were detected among the individuals of the seven populations of screwworm analysed. The average of nucleotide diversity was 0.92%. The nucleotide divergence estimates between pairs of haplotypes ranged from 0.3% to 2.7%. The analysis of the geographical distribution among the observed haplotypes suggests that the sampled populations probably belong to a single evolutionary lineage interconnected by reduced gene flow. The RAPD-PCR technique was used to detect genetic polymorphism and to select genetic markers to discriminate seven populations, including one from northern Argentina. In general, results of both mitochondrial, RAPD analysis and allozymes are concordant in suggesting divergence among screwworm populations. The Esterase system was the most polymorphic (with ten alleles) and was polymorphic in all the studied populations. The genetic differentiation, Fst value, was Fst=0.214. The estimated rate of gene flow from the total sample of screwworm was low Nm=0.92. Our data show a great amount of genetic variability as revealed by isozymes. In addition, mitochondrial DNA and RAPD data on samples from the same region also showed great genetic variability. Considering these data, and the reports of low genetic variability in North and Central Americas, we suggest that the high varibility in the species in South America could indicate that the evolutionary history of the species involved the spreading of ancestral tropical populations through North America.

The complete sequence of the mitochondrial genome of the screwworm fly was determined in our laboratory. This genome, 16.022 bp in size, corresponds to a typical Brachycera mtDNA. The nucleotide composition of mtDNA is 77% AT-rich, reflected in the predominance of AT-rich codons in protein-coding genes. The identification of diagnostic restriction sites on the sequenced mitochondrial genome and the correlation with previous RFLP analysis were conducted. The complete sequence of this genome should allow the identification of species-specific genetic markers in the primary mtDNA sequence which will be useful for monitoring and controlling this pest.

2. SIT in Uruguay: Screwworm Eradication and the Need for Integration for a Successful Pest Management Program

Dr. Juan Cristina, Centro de Investigaciones Nucleares, Facultad de Ciences, Igua 4225, 11400 Montivideo, Uruguay

The Nuclear Research Center at the School of Sciences is the unique nuclear facility constructed and devoted to work with radiation and isotopes in Uruguay. SIT technology experience was gained working with the Chagas' disease vector, *Triatoma rubrovaria*, as a model (Cristina *et al.*, 1986; Salvatella *et al.*, 1987; Cristina *et al.*, 1985, 1984a,b,c). At the present time, working together with the Ministry for Cattle, Agriculture and Fisheries, it was possible to determine the importance of New World Screwworm control and/or eradication from Uruguay. This pest challenges the main export products of Uruguay. Regarding myiasis, four different species of Diptera have been found in Uruguay: *Chlochliomyia hominivorax, Chlochliomyia macellaria, Chrysomya albiceps* and *Dermatobia hominis. C. hominivorax* accounts for 87.2 % of all myiasis in Uruguay.

The general prevalence of the disease is 4.5% of bovines and 6.2% of ovines are affected. The death rate of affected animals is calculated to be 6.5% for bovines and 18.5% for ovines. Considering a population of 10 million cattle and 26 million ovine in Uruguay, 450,000 bovines and 1,612,000 ovines are affected each year. Total loses are estimated to be US$24 million per year.

Since no geographical barrier separates Uruguay from Brazil, integrated management is the only choice to successfully control this important pest. SIT against screwworm would be very beneficial for Uruguay.

3. Current Costs of the Screwworm Program

P. Kaiser, Chief of methods Development, Mexico-US Commision for the Eradication of Screwworm, USDA-APHIS, IS, SWP Region 5, POB 31490, Laredo, Texas, USA

The costs of the Screwworm Eradication Program in Central America were divided into 5 major areas: 1) Production Facility; 2) Transportation of Irradiated Pupae; 3) Dispersal Center; 4) Dispersal of Irradiated Pupae; 5) Program Overhead. Annual Production Facility costs were estimated to be $7.75 million dollars, or about $1,000 for every million files produced (does not include salaries of U.S. employees). Annual transportation costs, based on 7 pupal shipments/wk to Panama, were about $1.4 million. The operational costs of the Dispersal center in Jamaica were $48,000/yr (excluding salaries). The average annual dispersal costs for each aircraft was approximately $700,000 (7 dispersal AC required for 7 shipments/wk). Sources of Program Overhead costs were identified but not quantified.

4. Screwworm Eradication in North and Central America

P. Kaiser, Chief of methods Development, Mexico-US Commission for the Eradication of Screwworm, USDA-APHIS, IS, SWP Region 5, POB 31490, Laredo, Texas, USA

Highlights of the Screwworm Eradication Program in North and Central America were presented. The Southeastern Program (1957-59) and the Southwestern Program (1962-66) eradicated screwworm from the U.S. The Mexican Program, which was started in 1972,

eliminated screwworm from this large and geographically diverse country in 1991. In '90-91 there was a screwworm outbreak in Libya, and in '92-93 several outbreaks occurred in Mexico; all outbreaks were successfully controlled. Eradication programs were successful in the following Central American countries: Belize ('93), Guatemala ('93), El Salvador ('94), Honduras ('95), Nicaragua ('98), Costa Rica ('00), Panama (projected '01). An eradication program was initiated in Jamaica in Aug. '99. Major benefits of the Screwworm Program are: 1. Livestock producer benefits; 2. Human health benefits; 3. Pet and wildlife health benefits; 4. Environmental benefits. Annual producer benefits for the U.S., Mexico and Central America are $853 million, $314 million and $87.8 million, respectively.

5. Genetic Sexing System for Anopheles albimanus

P. Kaiser, Chief of methods Development, Mexico-US Commission for the Eradication of Screwworm, USDA-APHIS, IS, SWP Region 5, POB 31490, Laredo, Texas, USA

In 1996 a genetic sexing system was developed for the Central American malaria vector, *An albimanus.* X-rays were used to induce chromosomal rearrangements that linked an insecticide resistant gene to the Y chromosome. Six strains were established, and the recombination varied from 6-27%. Several of these strains irradiated to induce inversions that would "cover" the resistant gene and the translocation breakpoint. Four strains were isolated that reduced recombination to 0.2-2.3%. The strain, In(2R)[T(Y;2R)3]2, later termed "Macho", was used in 1978-79 in a successful SIT pilot program in El Salvador, where 1 million sterile males was produced and released daily over a 150 sq km area.

6. Lucilia cuprina *Sexing Systems*

R. Mahon, CSIRO Division of Entomology, c/o Institute Haiwan, POB 520, 86009 Klung, Johor, Malaysia.

Sexing systems have been developed for the Australian sheep blowfly *Lucilia cuprina.* The first system consisted of Y-autosome translocation strain where females were homozygous susceptible (+ / +) at the dieldrin resistance locus, *Rdl,* while males were heterozygous (*Rdl / +)* and therefore resistant to dieldrin. When first instar larvae were treated with an appropriate concentration of the insecticide, females died. While functional in the laboratory, males were found to be poorly competitive in the field. Further development of the Y-Autosome system led to a Y-5,3 translocation bearing strain where females were homozygous for eye colour mutants (and thus blind) while males were heterozygous and thus sighted. This strain was used in genetic control trials and proved to be effective in imparting genetic load into native populations. However, under mass rearing conditions, recombination within the elements involved in the translocation produced individuals that were more fertile than flies carrying the Y-5,3 translocation, and these rapidly increased in frequency. A further construct was made that included a temperature sensitive mutation (enabling female elimination), and pericentric inversions, that increased the genetic load imparted when released into the field, and also protected against recombination that might lead to the breakdown of the rearrangement.

7. Population Genetics of Old World Screwworm

R. Mahon, CSIRO Division of Entomology, c/o Institute Haiwan, POB 520, 86009 Klung, Johor, Malaysia.

Concern that a single strain of OWS may not be effective in a SIT program against all populations of the pest led to a search for sibling species within the known range of the species. Various techniques were employed (allozyme variation, polytene chromosomal variation, cuticular hydrocarbon analyses and laboratory hybridisation) to examine the genetic variability and compatibility of crosses between samples from numerous localities throughout the range of the species. Sites samples included South Africa, Zimbabwe in Southern Africa, Oman in the Middle East, several sites in Malaysia and Indonesia in South East Asia and Papua New Guinea. No major discontinuities that might indicate the presence of sibling species were found. Indeed, the allozyme study concluded that the degree of genetic differentiation was remarkably small. Populations from the extremes of the range (South Africa and Papua New Guinea) and the intervening populations were so closely related that either significant levels of gene flow must be present to maintain the homogeneity of the loci sampled (highly unlikely) or the species has "recently" expanded its range to its present distribution.

8. Research on Genetic Sexing and Population Genetics of Screwworm at Lincoln, Nebraska

S. R. Skoda, USDA-ARS Midwest Livestock Insects Research Laboratory, Department of Entomology, UNL, Lincoln, Nebraska, USA

Responsibility for screwworm research at the USDA-ARS-Midwest Livestock Insects Research Unit (MLIRU) at Lincoln, NE began in 1991. A state-of-the-art biosecurity facility, completed in 1994, is where all screwworms are reared. Of the four scientists in the MLIRU, two work on screwworm. Iso-enzyme analyses are capable of identifying screwworm samples and showing genetic variability in screwworm populations but it is a cumbersome technique. PCR-RFLP analysis of mtDNA indicated that this technique was valuable for identifying screwworm from other species but was not well suited for quantifying genetic variability. RAPD-PCR analysis showed value in identifying screwworm from other species and promise for quantifying genetic variability across the geographic range of screwworm. Monoclonal antibodies against all stages of screwworm were isolated and a procedure established that allows the development of a test-kit, for use in the field, which is capable of identifying all stages of screwworm from other fly species. Techniques have been developed for the cryopreservation of screwworm eggs, allowing for efficient genome preservation. Finally, all extant screwworm strains at MLIRU were bioassayed, levels of insecticide tolerance were determined, and selection is progressing to establish insecticide resistance in one strain. Once resistance is established, classical genetic techniques (i.e. translocation of resistance 'gene' to the Y-chromosome) will be used to develop a genetic sexing strain. Concurrently, we intend to screen screwworm for temperature sensitivity (temperature sensitive lethals) and, if found, use those 'genes' in a classical approach to developing a genetic sexing strain. The most competitive genetic sexing strain(s) would be evaluated for use in the screwworm eradication program.

ANNEX 5

CONSULTANTS' MEETING ON

Genetic Sexing and Population Genetics of Screwworms

7-11 August, 2000
Vienna International Center, IAEA- Building A, Room 2210

Agenda

Monday, 7 August *INTRODUCTION AND PRESENTATIONS*

09:00 Opening **Mr. Robinson**
09:15 Introduction **Mr. Robinson**
09:45 Administrative matters **Mr. Robinson**
10:00 *Break*
10:30 The Insect and Pest Control Sub-Programme **Mr. Hendrichs**
11.00 Screwworm Activities within the IPC **Mr. Feldmann**
11:30 Entomology Laboratory Activities **Mr. Robinson**
12.15 *Lunch*
14:00 Genetic Sexing Experiences with Medfly, Lessons for Screwworm **Mr. G. Franz**
14:45 Research on Genetic Sexing and Population Genetics of Screwworm at Lincoln, Nebrsaka **Dr. S. Skoda**
15.30 *Break*
15.45 SIT in Uraguay: Screwworm Eradication and the Need for Integration for a Successful Pest management Programme **Dr. J. Cristina**
16.30 Discussion
17.00 Closure
17.30 Cocktails

Tuesday, 8 August *PRESENTATIONS*

09:00 Contribution of molecular markers to characterize genetic variability in Brazilian Screwworm fly. **Dr. Axeredo-Espin**
09:45 Population genetics of Old World Screwworm **Dr. Mahon**
10:15 *Break*
10:45 Genetic Sexing in *Lucilia cuprina* **Dr. Mahon**
11:15 Screwworm Eradication Program in Central America and how a Genetic Sexing Strain May Affect Program Costs; **Dr. Kaiser**
11:45 Genetic Sexing in *Anopheles albimanus*. **Dr. Kaiser**
12:15 Discussion of presentations
12:30 Departure for Seibersdorf with lunch *en route*
14:00 Visit to the Entomology Unit
15:30 *Break*
16:00 Information on the formulation of a Co-ordinated Research Project (CRP)
16:45 Departure for Vienna

Wednesday, 9 August *GENERAL DISCUSSIONS ON THE CRP*

09:00 Background Situation Analysis
09:45 Overall Objective
10.30 *Break*
11:00 Specific Research Objectives
11:45 Expected Research Outputs
12:30 *Lunch*
14:00 Discussion of the logical framework
15:30 *Break*
16:00 Continuation
Heurigen Evening-

Thursday, 10 August *DRAFTING OF THE CRP*

09:00: Drafting of the CRP
12:30 *Lunch*
14.00 Drafting of the CRP
17:00 Wrap-up

Friday, 11 August *FINAL DRAFT AND REPORTING*

09:00 Final Draft
12:30 *Lunch*
13:00 Presentation of Conclusions and Recommendations
16:00 Closing